

growth factor (VEGF) has been shown to accelerate re-endothelialization at incisions in balloon-injured arteries. Since endothium-derived nitric oxide smooth muscle growth, we tested whether VEGF enhances nitric oxide cell cells. Experiments were performed with primary cultures of human cell cells (HMEC) and with endothelium-rich rat aortic rings. Nitric oxide production was assessed by RT-PCR and Western blot analyses, and NOS (N of cGMP in HMEC and endothelium-dependent relaxations in aortic VEGF to VEGF165 (100 ng/ml) for 48 h lead to an increase in NOS in and in the basal level of cGMP (2.2 \pm 0.3 fold) whereas that evoked by (SNP) was unaffected. VEGF treatment increased in a concentration-OS II mRNA levels within 2 h which remained elevated for the next 48 h. 1 of VEGF on NOS II mRNA was abolished by protein tyrosine kinase and estrogen A) and was not prevented by the inhibitor of transcription sure of aortic rings to VEGF for 7 h potentiated endothelium-dependent home whereas those to SNP were unaffected. Increased levels of NOS II are found in VEGF-treated aortic rings. These findings indicate that VEGF expression in native and cultured endothelial cells. This effect is mediated nitric oxide-dependent pathway(s) and seems to be due to the stabilization of new generation of endothelial nitric oxide may contribute to the protective non-injured arterial thickening.

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synthase (nNOS) has been localized to the inner membrane of skeletal sarcoplasmic reticulum. nNOS is mallocalized to the cytosol and has been gross of the sarcolemma. A protein inhibitor of nNOS (PIN) which specifically inhibits nNOS dimerization has recently been cloned. The 89 residue PIN regulates the biochemical activity of nNOS. We have, therefore, examined, by Northern blot analysis, the distribution and regulation of PIN transcript in skeletal muscles under physiological conditions where nNOS expression has been shown to be regulated. PIN was detected in limb muscles of normal rats but the highest levels of PIN were detected in soleus muscle which has the lowest nNOS expression. In the OS and PIN expressions were elevated in embryonic combined with adult soleus muscle. Significant developmental regulation, in embryonic muscle (20 mg/kg E coli), OS and PIN expressions were elevated in limb muscles within 12 hours of denervation. We further examined the levels of PIN and nNOS in the mouse myoblast cell line C2C12. In growing myoblasts, significant PIN mRNA expression was detected. High PIN expression was maintained following the induction of myoblast fusion and in the mature muscle. These data indicate that PIN is expressed in various skeletal muscles in vivo and that its expression correlates with that of nNOS. Moreover, PIN mRNA is regulated under physiological conditions such as denervation.

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200c synthase (iNOS) is expressed in a variety of inflammatory disorders in system, and iNOS expression is regulated at the transcriptional level. We earlier demonstrated that the synergistic induction of the iNOS gene elicited by TNF- α and IFN- γ activates NF- κ B and IRF-1 in macrophages, and these transcription factors bind to the κ B and the IRF sites in the iNOS promoter to induce iNOS. We now show that IRF-1 and NF- κ B interact with each other. Co-immunoprecipitation studies show that IRF-1 and NF- κ B are bound to each other only in stimulated cells. *In vitro* experiments show that IRF-1 and NF- κ B interact while binding to DNA target region. The proteins bound to a κ B site include not only NF- κ B but also IRF-1. Similarly, the proteins bound to an IRF site include not only IRF-1 but also NF- κ B. These results demonstrate the existence of a physical interaction between these two transcription factors *in vivo*. To explore the functional consequences of the interaction between NF- κ B and IRF-1, we examined their ability to affect the structure of the iNOS promoter. We found that IRF-1 and NF- κ B alter the structure of the iNOS transcription factors binding site, and the DNA structure at the site of binding to NF- κ B is altered by IRF-1. These results suggest that the interaction between NF- κ B and IRF-1 is important for the regulation of iNOS expression.

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nitric oxide (NO), a vasodilator involved in the regulation of pulmonary vascular tone, is synthesized by a class of enzymes, NO synthases (NOS). We have previously shown that adenovirus-mediated overexpression of the calcium-dependent type III NOS in rat lungs reduces acute hypoxic pulmonary vasoconstriction. To quantitate the onset and duration of NO production following NOS gene transfer, we measured censored NO by chemoluminescence in rats infected with adenovirus expressing the calcium-dependent type III NOS (4×10^8 pfu/ml, $n=7$), type II NOS ($n=8$), or control virus expressing no transgene (AdRRS, $n=7$). Censored NO was increased in NOS II-infected rats compared to NOS-III-infected rats at 24 h (55 ppb vs 47 ppb), 4 d (52 ppb vs 36 ppb), and 7 d (31 ppb vs 22 ppb), but no longer at 10 d. The levels of NO in AdRRS-infected rats were significantly lower at all time points (19±2 ppb at 24 h, 6±3 ppb at 4 d and 7±1 ppb at 7 d). To investigate whether increased pulmonary NO production after NOS II gene transfer was associated with greater inhibition of hypoxic pulmonary vasoconstriction, mean pulmonary artery pressure (PAP, mmHg) was measured during acute hypoxia ($PO_2 = 0.10$, 25 mm) in rats 4 d after infection with NOS II ($n=7$), NOS III ($n=8$), or control virus ($n=6$). Acute hypoxia decreased PAP from 19±4 to 21±5 mmHg in NOS II-infected rats compared to 23±2 mmHg in NOS III-infected and 28±2 mmHg in control virus-infected rats with no significant effect on systemic blood pressure. Thus, pulmonary NOS II gene transfer significantly increases pulmonary censored NO production for at least 7 days and is associated with a greater inhibition of acute hypoxic pulmonary vasoconstriction. Single intrapulmonary NOS II gene transfer may be a promising therapy for pulmonary hypertension.

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Multiple studies suggest that lipid-endoplasmic mechanisms contribute to the development of cardiovascular disease in diabetes. Under conditions of sustained hyperglycemia, nonenzymatic glycosylation and oxidation of proteins and lipids results in the irreversible formation of Advanced Glycation Endproducts (AGEs) which accumulate in diabetic plasma and tissues. AGEs interact with cellular receptors such as RAGE, the Receptor for AGEs, and induce vascular cell dysfunction. The extracellular portion of RAGE (one V-type and two C-type immunoglobulin domains) is a soluble fragment (sRAGE) which can passively bind AGEs and block their interaction with their cell-surface receptor. We previously demonstrated a 3.7-fold increase in atherosclerotic lesion area in streptozotocin-treated apolipoprotein E deficient mice vs. controls, with enhanced accumulation of AGEs and increased expression of RAGE in the vasculature by immunohistochemistry. To test if blockade of AGE-RAGE would suppress accelerated atherosclerosis, diabetic apo E deficient mice were treated for six weeks with sRAGE (200 μ g/day, intraperitoneally) or equivalent mouse serum albumin (400 μ g/day MSA). Mean lesion area was decreased 1.8-fold ($P=0.016$) in mice treated with sRAGE (150,000 \pm 18,549 μ m²) vs MSA (271,000 \pm 16,721 μ m²). No difference was observed at a low dosage of 30 μ g/day; enhanced anti-atherogenic effects were observed with higher doses of 30–40 μ g/day. Serum albumin glucose and HbA_{1c} levels revealed persistent hyperglycemia in both groups. There were no differences in levels of total cholesterol and triglycerides, and FPLC analyses yielded identical lipid profiles. Taken together, these data suggest that enhanced AGE-RAGE interaction likely plays a critical role in the pathogenesis of accelerated atherosclerosis in diabetes.

Essential Role of Endothelial Nitric Oxide Synthase in Angiogenesis *In Vivo*

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